Environmental Health Perspectives
Vol. 63, pp. 149-153, 1985

Relationship of Aluminum to Alzheimer's Disease

by Daniel P. Perl*

Alzheimer's disease is a progressive degenerative brain disease of unknown etiology, characterized by the development of large numbers of neurofibrillary tangles and senile plaques in the brain. Aluminum salts may be used experimentally to produce lesions which are similar, but not identical, to the neurofibrillary tangle. Although some studies have reported increased amounts of aluminum in the brains of Alzheimer's disease victims, these bulk analysis studies have been difficult to replicate and remain controversial. Using scanning electron microscopy with X-ray spectrometry, we have investigated this question on the cellular level. We have identified abnormal accumulations of aluminum within neurons derived from Alzheimer's disease patients containing neurofibrillary tangles. Similar accumulations have been detected in the numerous neurofibrillary tangle-bearing neurons seen in the brains of the indigenous native population of the island of Guam who suffer from amyotrophic lateral sclerosis and parkinsonism with dementia. Epidemiologic evidence strongly suggests a causal role for local environmental conditions relating to availability of aluminum, calcium, and magnesium. In view of the fact that a major consequence of acid rain is the liberation of large amounts of aluminum in bioavailable forms, concerns are raised about possible human health risks of this environmental phenomenon.

Introduction

Despite the fact that the histologic and clinical features of Alzheimer's disease have been documented and extensively studied over the past century, we still do not have a clear concept of this disorder's etiology or pathogenesis. For the neuropathologist, Alzheimer's disease is a condition that is characterized clinically by progressive cognative dysfunction and pathologically by the development of large numbers of neurofibrillary tangles and senile or neuritic plaques in the neocortex and hippocampus (*1*). Alzheimer's disease represents a progressive degenerative disease of the central nervous system of unknown etiology.

In our laboratory, we have studied the potential role of aluminum in the pathogenesis of the neurofibrillary tangle, an important component in the histologic changes seen in Alzheimer's disease. In this paper, we will discuss the association of aluminum to Alzheimer's disease and to other conditions in which neurofibrillary tangles are encountered, as well as the data, both for and against, of a causal link related to the neurotoxicity of this element. Finally, we will discuss the implications of changes in geochemistry and ecosystems associated with acid rain, and their potential implications to altered risks for aluminum neurotoxicity.

*Department of Pathology, University of Vermont College of Medicine, Burlington, VT.

Aluminum and Alzheimer's Disease

The potential association of aluminum and Alzheimer's disease began with observations made in 1965, by Klatzo and co-workers (*2*) and by Terry and Peña (*3*), of the experimental induction of neurofibrillary degeneration in rabbits following exposure of the central nervous system to aluminum salts. These workers and others (*4,5*) have described prominent, widespread, neurofilamentous accumulations which developed in neuronal perikarya following the introduction of aluminum salts directly onto the central nervous system parenchyma or into the cerebrospinal fluid. These experimentally induced neurofilamentous changes were orignally considered to be comparable to human neurofibrillary tangles.

However, criticism soon began to be raised concerning the use of the aluminum-induced neurofilamentous accumulations as an experimental model of the human neurofibrillary tangle (*3,6*). At the time, the human neurofibrillary tangle was considered by most workers in the field to be an altered neurotubule (the so-called twisted tubule) (*7*), whereas the aluminum-induced tangles were composed of straight neurofilaments. Subsequent investigation of this issue led to the acceptance of the concept of the human neurofibrillary tangle being composed of a pair of helically wound neurofilaments (*8,9*), the so-called paired-helical filament, thus provid-

ing a closer link to the aluminum model.

Currently, unresolved issues exist related to shared versus separate antigenic determinants between normal neurofilaments and paired helical filaments (*10–14*). At present, mechanisms for the formation of the unique paired helical configuration of the cytoskeletal elements seen in man's neurofibrillary tangle are not clearly established. Although the precise biochemical nature of the subunits of the paired helical filaments is currently uncertain, structural and antigenic evidence exists which suggests that an altered neurofilament is a likely candidate (*15–17*). An inherent resistance of the paired-helical filaments to become solubilized following harsh proteolytic treatment has significantly hampered progress in this field (*14*). Until the nature of the basic components of the paired helical filaments are known, along with the mechanism for its unique structural configuration, not encountered naturally or experimentally, in animals other than man, these concerns will remain unresolved.

The existence of the rabbit animal model stimulated Crapper-McLachlan and colleagues to investigate the aluminum content of brain tissues of patients with Alzheimer's disease. These workers reported (*18,19*) significantly increased levels of brain aluminum content, particularly in those regions of the brain containing large numbers of neurofibrillary tangles. Crapper-McLachlans' work has been difficult to replicate and this subject remains controversial (*20–22*). All of these studies employ assay techniques which are inherently tissue destructive (atomic absorption spectrometry and neutron activation analysis) and also require relatively large bulk samples. In a situation of focal accumulations of an element in association with certain cellular lesions, dilution of the element of interest within the relatively large total sample being analyzed may result in an inability to detect significant differences between affected tissues and uninvolved controls.

Scanning Electron Microscopy–X-Ray Spectrometry Studies

In our laboratory, we have developed highly sensitive techniques, through the use of scanning electron microscopy in conjunction with energy dispersive x-ray spectrometry, for the analysis of trace element constituents of the nervous system at the cellular level of resolution (*23–25*). The tissues being analyzed are prepared as frozen sections of formalin fixed brain samples. Cells of interest are located through examination of the secondary electron surface images or the appearance of back-scattered electron images obtained from silver-stained sections. The electron beam is then focussed to approximately one micron in diameter and this minute portion of the section is irradiated with electrons, producing X-rays. The X-rays that are emitted are characteristic in their energy levels for the elements contained within the portion of the tissue being hit by the electron beam. By collecting and analyzing the X-rays emitted from selected portions of individual neurons, we can provide information about elemental content with rather precise cellular localization.

Using these techniques, we have identified intraneuronal accumulations of aluminum in association with neurofibrillary tangle formation in the hippocampal neurons of brain tissues obtained from patients with Alzheimer's disease (*23,26*). Adjacent tangle-free neurons of these cases, or of age-matched controls, failed to show a similar degree of intraneuronal aluminum accumulation. The aluminum accumulations appear to be confined to the nuclear region of the cell and are not found in the perikaryal cytoplasm, the neurofibrillary tangle itself, or the adjacent neuropil.

Studies of High-Risk Foci

Although a pathologic hallmark of Alzheimer's disease, neurofibrillary tangles are found in association with a wide variety of other neurologic disorders (*27*). In an attempt further to evaluate intraneuronal aluminum content in association with neurofibrillary tangle formation, we have studied the remarkable tendency towards the development of this form of neuronal pathology shown by the Chamorro people native to the island of Guam. This group of indigenous natives show an inordinately high tendency to develop certain neurodegenerative disorders; namely, a form of parkinsonism associated with severe dementia and amyotrophic lateral sclerosis (ALS). Neuropathologic examination of the brains of parkinsonism with dementia cases from Guam shows a profound loss of the pigmented neurons of the substantia nigra as well as severe widespread neurofibrillary tangle formation (*28*). The central nervous system of cases of ALS from Guam show the classic features of motor neuron degeneration as well as extensive neurofibrillary tangle formation, a feature rarely encountered in cases of ALS seen elsewhere in the world (*29,30*).

In collaboration with the NIH-Laboratory of Central Nervous System Studies, we have been engaged in the study of the trace element constituents of the tangle-bearing neurons encountered in the Chamorro natives of Guam. Using our scanning electron microscopy–X-ray spectrometry probe techniques, we have clearly demonstrated that aluminum selectively accumulates within the tangle-bearing neurons of the Guamanian native affected with parkinsonism-dementia and ALS (*26,31*). The aluminum-related X-rays generated from probe sites of the tangle-bearing neurons of these cases were 3–4 times greater than those obtained from tangle-free Guamanian control specimens. The dramatic accumulation of aluminum in the tangle-bearing neurons of the Guamanian Chamorro has recently been independently confirmed using a different X-ray spectrometry imaging approach (*32*).

Table 1 provides data related to X-ray probe sites taken on tangle-bearing and tangle-free hippocampal neurons of cases of Alzheimer's disease, Guamanian ALS and parkinsonism-dementia and appropriate con-

Table 1. Aluminum-related x-rays collected from hippocampal neurons.

Case	Age, yr	Neurofibrillary tangles	X-ray counts (± 1 SE)		
			Nuclear region	Perikaryal cytoplasm	Adjacent neuropil
Alzheimer's Disease	88	+	160.5 ± 14.3	118.5 ± 23.1	48.6 ± 14.0
Alzheimer's Disease	83	+	168.9 ± 14.8	104.6 ± 16.6	27.8 ± 11.7
U.S. control	88	−	75.8 ± 11.9	65.3 ± 12.8	86.8 ± 30.0
U.S. control	82	−	64.9 ± 12.1	89.4 ± 14.4	61.8 ± 33.0
Guam ALS	67	+	299.1 ± 28.7	330.4 ± 30.4	179.1 ± 43.8
Guam PD	66	+	232.1 ± 23.8	226.8 ± 34.6	99.4 ± 24.1
Guam control	48	−	106.8 ± 17.4	117.9 ± 17.4	109.3 ± 29.4
Guam control	65	−	100.9 ± 17.2	90.1 ± 14.4	60.3 ± 14.8

trols. The sections were cut on a cryostat at 20 μm from blocks of formalin-fixed tissue. The sections were mounted unstained on pure carbon stubs, air-dried, and lightly carbon coated. Regions of the hippocampus with numerous tangle-bearing neurons were identified in serially cut sections which were stained with the Bielschowsky silver technique and viewed by light microscopy. Using identical conditions of specimen preparation, X-ray generation and data collection, 20 neurons from the CA_1 region of the hippocampus were subjected to multipoint X-ray probe analysis. The data provided gives the aluminum-related X-ray counts (± 1 S.E.) following computer-assisted background subtraction within a 150-eV window centered around the K-alpha of aluminum.

It should be pointed out that these techniques permit us to simultaneously evaluate several other elements within the tissues being probed. In these studies, aluminum represents the only element which is consistently altered in relationship to the presence, in a neuron, of a neurofibrillary tangle. Many of the cases have shown increased calcium-related X-ray signals in the tangle-bearing neurons (*26*). Some cases, however, have failed to demonstrate a similar accumulation. In contrast to the tightly bound nature of the aluminum, much of intracellular calcium is lost following the death of the individual and in the fixation of the specimen. Accordingly, we are presently unable to fully interpret the calcium-related data.

Over the past 35 years, research interest in the phenomenon of neurodegenerative disease of epidemic proportions among the Guamanian natives has centered primarily on attempts to determine the underlying mechanisms responsible for motor neuron degeneration. As D. Carleton Gajdusek has written (*33*), "Discovery of its cause and pathogenesis in these intensely affected populations will surely contribute to a better understanding of the disease (ALS) elsewhere." It has been our belief that the remarkable tendency of the natives living on Guam to develop large numbers of neurofibrillary tangles represents a similar opportunity with respect to Alzheimer's disease.

Evaluation of a large amount of epidemiologic evidence strongly suggests that certain environmental factors may play an important etiologic role in the neurodegenerative phenomena so prevalent on Guam (*34,35*). Important in this consideration are observations made of two additional foci in the western Pacific both of which show a similar high incidence of ALS and parkinsonism-dementia, that is, the Kii peninsula of southern Japan (*36*) and the Auyu and Jakai people of southwestern New Guinea (*37*). Careful evaluation of these populations fails to reveal shared genetic constituencies and environmental factor/s common to all three high-risk foci have been sought.

It has been noted that all three areas have an aluminum-rich bauxite soil in association with markedly low levels of calcium and magnesium in the local garden soil and water supplies (*38,39*). Until recently, all three foci remained isolated with extremely limited access to imported foodstuffs. It is of particular interest that, over the past two decades, Guam has witnessed a dramatic drop in the incidence of both ALS and parkinsonism-dementia (*40*). Indeed, instead of having a fifty-fold higher risk of developing ALS, as existed 30 years ago, the Guamanian Chamorro now has a risk that is only a few times higher than that of the U.S. resident. It is unclear whether this dramatic change in incidence data is related to the coincident influx, during this same period of time, of many of the commercial products associated with "westernization." However, there can be virtually no doubt that this relatively abrupt change in incidence strongly suggests that the environment plays an important causal role in these phenomena.

Aluminum, Alzheimer's Disease and the Environment

It is difficult to speculate on the implications of the data obtained from the Guam focus to an understanding of Alzheimer's disease pathogenesis, as seen elsewhere in the world. Aluminum is the most abundant metallic element, comprising 5% of the earth's crust. For the most part, aluminum is largely present in insoluble, and thus biologically unavailable, forms. Relatively little is known about patterns of long-term aluminum exposure in patients who eventually develop Alzheimer's disease. Case-control studies have not detected any significantly increased use of aluminum-containing antacids (*41*). Data related to the tendency for subsequent development of neurofibrillary tangles or outright Alzheimer's

disease in individuals with industrial exposure to aluminum have not, to our knowledge, been published.

Despite its natural abundance, it is clear that aluminum can be a potent neurotoxin to certain mammalian species. The means by which the element gains access to the tangle-bearing neurons seen in the brains of Alzheimer's disease victims or of the affected Chamorro natives currently remains unknown. Under normal conditions, relatively little aluminum is absorbed by the gastrointestinal system following oral exposure (*42*) and an extremely small amount ends up residing in nervous system tissues. We assume that under conditions of intraneuronal aluminum deposition some form of blood-brain barrier has been breached. We still do not know if changes in factors such as aluminum speciation may play an important role in ultimately determining neuronal bioavailability of aluminum encountered in our environment.

Considering the above, it is of interest, and perhaps of some concern, that we note that one of the principle effects of acid rain is to leach large amounts of aluminum from the soil due to its lack of buffering capacity. Evidence has been presented suggesting that an important biologic effect of acid rain is to increase the bioavailability of aluminum to both flora and fauna, with profound toxic effects. How high up the biologic ladder this concept may extend can only be speculated upon.

It should be remembered that, although aluminum is one of the most ubiquitous elements in our environment, it has, until relatively recently, existed predominantly in forms unavailable to man and most other biologic species and is thus nontoxic. Accordingly, in an evolutionary sense, little has been required, in terms of natural barrier systems or detoxifying mechanisms, to deal with this substance. With the relatively recent appearance of acid rain, there has been a dramatic increase in the amount of aluminum appearing in biologic ecosystems with dramatically destructive effects on fish and plant-life species. It is clear that these species have been unable to adapt successfully to these environmental changes. Whether man will find himself in a similar vulnerable state as regards changes in aluminum bioavailability, can only be speculated upon. It is clear that the potential magnitude of the problem requires urgent further scientific study.

REFERENCES

1. Corsellis, J. N. N. Aging and the dementias. In: Greenfield's Neuropathology, 3rd ed. (W. Blackwood and J. A. N. Corsellis, Eds.), Edward Arnold, Edinburgh, 1976, pp. 796.
2. Klatzo, I., Wisniewski, H., and Streicher, E. Experimental production of neurofibrillary degeneration. I. Light microscopic observations. J. Neuropath. Exptl. Neurol. 24: 187–199 (1965).
3. Terry, R. D., and Penã, C. Experimental production of neurofibrillary degeneration. 2. Electron microscopy, phosphatase histochemistry and electron probe analysis. J. Neuropath. Exptl. Neurol. 24: 200–210 (1965).
4. Wisniewski, H. M., Narkewicz, O., and Wisniewski, K. Topography and dynamics of neurofibrillary degeneration in aluminum encephalopathy. Acta Neuropathol. 9: 127–133 (1967).
5. Crapper, D. R., and Dalton, A. J. Alterations in short-term retention, conditioned avoidance response, acquisition and motivation following aluminum induced neurofibrillary degeneration. Physiol. Behav. 10: 925–933 (1973).
6. Terry, R. D. Electron microscopic studies of Alzheimer's disease and of experimental neurofibrillary tangles. In: The Central Nervous System (O. T. Bailey and D. E. Smith, Eds.), Williams & Wilkins, Baltimore, 1968, p. 213.
7. Wisniewski, H. M., Terry, R. D., and Hirano, A. Neurofibrillary pathology. J. Neuropath. Exptl. Neurol. 29: 163–176 (1970).
8. Kidd, M. Paired helical filaments in electron microscopy of Alzheimer's disease. Nature 197: 192–193 (1963).
9. Wisniewski, H. M., Narang, H. K., and Terry, R. D. Neurofibrillary tangles of paired helical filaments. J. Neurol. Sci. 27: 173–181 (1976).
10. Ihara, Y., Abraham, C., and Selkoe, D. J. Antibodies to paired helical filaments in Alzheimer's disease do not recognize normal brain proteins. Nature 304: 727–730 (1983).
11. Grundke-Iqbal, I., Iqbal, K., Tung, Y.-C., and Wisniewski, H. M. Alzheimer paired helical filaments: immunochemical identification of polypeptides. Acta Neuropathol. 62: 259–267 (1984).
12. Wang, G. P., Grundke-Iqbal, I., Kascsak, R. J., Iqbal, K., and Wisniewski, H. M. Alzheimer neurofibrillary tangles: monoclonal antibodies to inherent antigen(s). Acta Neuropathol. 62: 268–275 (1984).
13. Bignami, A., Selkoe, D. J., and Dahl, D. Amyloid-like (Congophilic) neurofibrillary tangles do not react with neurofilament antisera in Alzheimer's cerebral cortex. Acta Neuropathol. 64: 243–250 (1984).
14. Selkoe, D. J., Ihara, Y., and Salazar, F. J. Alzheimer's disease: insolubility of partially purified paired helical filaments in sodium dodecyl sulfate and urea. Science 215: 1243–1245 (1982).
15. Anderton, B. H., Breinburg, D., Downes, M. J., Green, P. J., Tomlinson, B. E., Ulrich, J., Wood, J. N., and Kahn, J. Monoclonal antibodies show that neurofibrillary tangles and neurofilaments share antigenic determinants. Nature 298: 84–86 (1982).
16. Gambetti, P., Autulio-Gambetti, L., Perry, G., Schecket, G., and Crane, R. C. Antibodies to neurofibrillary tangles of Alzheimer's disease from human and animal neurofilament fractions. Lab. Invest. 49: 430–435 (1983).
17. Gambetti, P., Shecket, G., Getti, B., Hirano, A., and Dahl, D. Neurofibrillary changes in human brain. An immunocytochemical study with a neurofilament antiserum. J. Neuropath. Exptl. Neurol. 42: 69–79 (1983).
18. Crapper, D. R., Krishnan, S. S., and Dalton, A. J. Brain aluminum distribution in Alzheimer's disease and especially neurofibrillary degeneration. Science 180: 511–513 (1973).
19. Crapper, D. R., Krishnan, S. S., and Quirrkat, S. Aluminum, neurofibrillary degeneration and Alzheimer's disease. Brain 99: 67–80 (1976).
20. Markesbury, W. R., Ehmann, W. D., Hossain, T. I. M., Allauddin, M., and Goodin, D. T. Instrumental neutron activation analysis of brain aluminum in Alzheimer's disease and aging. Ann. Neurol. 10: 511–516 (1981).
21. McDermott, J. R., Smith, A. I., Iqbal, K., and Wisniewski, H. M. Brain aluminum in aging and Alzheimer disease. Neurology 29: 809–814 (1979).
22. Trapp, G. A., Miner, G. D., Zimmerman, R. L., Mastri, A. R., and Heston, L. L. Aluminum levels in brain in Alzheimer's disease. Biol. Psychiat. 13: 709–718 (1978).
23. Perl, D. P., and Brody, A. R. Alzheimer's disease: x-ray spectrometric evidence of aluminum accumulation in neurofibrillary tangle-bearing neurons. Science 208: 297–299 (1980).
24. Perl, D. P., and Brody, A. R. Detection of aluminum of SEM-x-ray spectrometry within neurofibrillary tangle-bearing neurons of Alzheimer's disease. Neurotoxicology 1: 133–137 (1980).
25. Perl, D. P. Detection of aluminum in nervous system tissues by scanning electron microscopy. In: Aluminum Analysis in Biological Materials (M. R. Wills and J. Savory, Eds.), Univ. Virginia Press, Charlottesville, VA, 1983, pp. 34–44.
26. Perl, D. P. Aluminum and Alzheimer's Disease: intraneuronal x-ray spectrometry studies. Banbury Report 15: Biological Aspects of Alzheimer's Disease, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1983, pp. 425–431.

27. Wisniewski, K., Jervis, G. A., Moretz, R. C., and Wisniewski, H. M. Alzheimer neurofibrillary tangles in diseases other than senile and presenile dementia. Ann. Neurol. 5: 288–294 (1979).
28. Hirano, A., Malamud, N., and Kurland, L. T. Parkinsonism-dementia complex, an endemic disease on the island of Guam. II. Pathological features. Brain 84: 662–679 (1961).
29. Malamud, N., Hirano, A., and Kurland, L. T. Pathoanatomic changes in amyotrophic lateral sclerosis on Guam: special reference to the occurrence of neurofibrillary changes. Arch. Neurol. 29: 573–578 (1961).
30. Hirano, A., Arumugasamy, N., and Zimmerman, H. M. Amyotrophic lateral sclerosis: a comparison of Guam and classical cases. Arch. Neurol. 16: 357–363 (1967).
31. Perl, D. P., Gajdusek, D. C., Garruto, R. M., Yanagihara, R. T., and Gibbs, C. J., Jr. Intraneuronal aluminum accumulation in amyotrophic lateral sclerosis and parkinsonism dementia of Guam. Science 217: 1053–1054 (1982).
32. Garruto, R. M., Fukatsu, R., Yanagihara, R., Gajdusek, D. C., Hook, G., and Fiori, C. E. Imaging of calcium and aluminum in neurofibrillary tangle-bearing neurons in parkinsonism-dementia of Guam. Proc. Natl. Acad. Sci. (U.S.) 81: 1875–1879 (1984).
33. Gajdusek, D. C. Foci of motor neuron disease in high incidence in isolated populations of East Asia and the Western Pacific. In: Human Motor Neuron Diseases (L. P. Rowland, Ed.), Advances in Neurology, Vol. 36, Raven Press, New York, 1982, pp. 363–394.
34. Kondo, K. Motor neuron disease: changing population patterns and clues for etiology. In: Advances in Neurology, Vol. 19 (S. Schoenberg, Ed.), Raven Press, New York, 1978.
35. Reed, D. M., and Brody, J. A. Amyotrophic lateral sclerosis and parkinsonism-dementia on Guam, 1945–1972. I. Descriptive epidemiology. Am. J. Epidemiol. 101: 287–301 (1975).
36. Shiraki, H., and Yase, H. Amyotrophic lateral sclerosis in Japan. In: Handbook of Clinical Neurology (P. J. Vinken, and G. W. Bruyn, Eds.), North Holland, Amsterdam, 1975, Vol. 22, pp. 353–419.
37. Gajdusek, D. C., and Salazar, A. M. Amyotrophic lateral sclerosis and Parkinsonism syndromes in high incidence among the Auyu and Jakai people of West New Guinea. Neurology 32: 107–126 (1982).
38. Yase, Y. The pathogenesis of amyotrophic lateral sclerosis. Lancet ii: 292–296 (1972).
39. Gajdusek, D. C. Foci of motor neuron disease in high incidence in isolated populations of East Asia and the Western Pacific. In: Human Motor Neuron Diseases. (Advances in Neurology, Vol. 36) (L. P. Rowland, Ed.), Raven Press, New York, 1982, pp. 363–394.
40. Garruto, R. M., Yanagihara, R., and Gajdusek, D. C. Disappearance of high-incidence amyotrophic lateral sclerosis and parkinsonism-dementia on Guam. Neurology 35: 193–198 (1985).
41. Heyman, A., Wilkinson, W. E., Stafford, J. A., Helms, M. J., Sigmon, M. J., and Weinberg, T. Alzheimer's disease: a study of epidemiological aspects. Ann. Neurol. 15: 335–341 (1984).
42. Mayor, G. H., Keiser, J. A., Makdain, D., and Ku, P. K. Aluminum absorption and distribution: effect of parathyroid hormone. Science 197: 1187–1189 (1977).